Histopathologic changes after thermokeratoplasty for keratoconus

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Six patients had penetrating keratoplasty following thermokeratoplasty (TKP) at different time intervals. The standard temperature used was between 90° and 95° C. with constant saline irrigation. The corneal buttons were studied by light and electron microscopy. Immediate changes observed included epithelial necrosis, marked degeneration of keratocytes, and endothelial edema. After 24 hours, evidence of acute inflammation was noted in the subepithelial zone and superficial stroma, and the endothelial changes persisted. One month after TKP, there was evidence of cellular regeneration of all the corneal layers. Eight months later, regeneration of the endothelial cells, keratocytes, and epithelium was present. Regeneration of the epithelial basement membrane was defective.

Key words: keratoconus, thermokeratoplasty, temperature, epithelium, keratocyte, Descemet's membrane, endothelium.

Thermokeratoplasty (TKP) has only recently been advocated as a therapeutic alternative to contact lenses and penetrating keratoplasty in the treatment of keratoconus. Techniques and clinical evaluation of this procedure have been previously described.1-5 Histopathologic changes in animal eyes have also been reported.6 Histologic changes visible by light or electron microscopy have not been reported in human corneas with keratoconus, following TKP.

The purpose of our study was to observe tissue changes induced by TKP at different time intervals, in patients with keratoconus, and to determine the extent of regeneration of the epithelium and its basement membrane, Bowman's membrane, keratocytes, and endothelium.

Material and methods

Thermokeratoplasty was performed in six patients who subsequently required penetrating keratoplasty at different time intervals. A 3 mm metal probe (Frigitronics of Connecticut, Inc., Shelton, Conn.) was used in all patients, and the standard temperature was between 90° and 95° C. Normal balanced saline solution was used to irrigate the cornea at the time of application.
of the probe. Details of our technique have been described elsewhere.

In two patients, TKP was performed in the operating room prior to removal of the host cornea, to observe the immediate changes. Four patients had penetrating keratoplasty 24 hours, 1 month, 2½ months, and 8 months after TKP. The last three patients had penetrating keratoplasty because of marked corneal scarring and thinning, present before TKP was performed.

All corneal buttons were bisected. One half of each button was fixed in formalin and processed for light microscopy. Paraffin sections were stained with hematoxylin and eosin, periodic acid–Schiff stain (PAS), and Prussian blue. The other half was fixed in 2.5 per cent buffered glutaraldehyde. In two keratoconus cases, half of each corneal button was processed for scanning electron microscopy (SEM). In one, TKP had not been performed; the other button was removed one month after TKP.

Transmission electron microscopy (TEM) was performed on representative portions of each specimen. These were post-fixed in 1 per cent osmium tetroxide, dehydrated in ascending concentration of alcohols, embedded in Epon, and examined with the Siemens 101 electron microscope. Controls included 12 cases of untreated keratoconus, all of which were studied by light microscopy and two by TEM.

**Results**

Light microscopy in corneal buttons removed immediately after TKP disclosed areas of thinned and necrotic epithelium, with areas of bullous separation. Intracellular edema was present in the basal cell layer, and Bowman's membrane was focally absent. The keratocytes were edematous throughout the full thickness of the stroma (Fig. 1). Descemet's membrane displayed irregular thickness, with focal ruptures. The endothelium revealed nu-
The stromal keratocytes exhibit nuclear pyknosis (N) and marked necrosis of cytoplasmic organelles. (x9,600.)

The lamella epithelium shows moderate edema. Irregular stromal necrosis, partial absence of Bowman's layer (arrow), and aggregates of macrophages and polymorphonuclear leukocytes can be seen. (Toluidine blue; x640.)

clear swelling and numerous cytoplasmic vacuoles (Fig. 1), in contrast to a control specimen of keratoconus in which TKP had not been performed (Fig. 1, inset). Transmission electron microscopy revealed marked epithelial necrosis of nuclear and cytoplasmic organelles. Similar changes were evident in stromal keratocytes (Fig. 2), but were not observed in untreated (control) cases of keratoconus.
Histopathology after thermokeratoplasty

At 24 hours after TKP, the corneas disclosed aggregates of macrophages and polymorphonuclear cells in the subepithelial region (Fig. 3). Epithelial edema and bullae were noted and edematous changes in the keratocytes and endothelial cells were present. Scanning electron microscopy revealed endothelial edema with marked nuclear pyknosis and cytoplasmic blebs (Fig. 4). Involvement of the epithelium and endothelium was confirmed by transmission electron microscopy.

One month later, TEM disclosed irregular regeneration of the epithelium (Fig. 5), although its basement membrane and basal epithelial cells’ attachment complexes were lacking or defective. The basal cells also displayed considerable edema and degeneration of cytoplasmic organelles (Fig. 5), in contrast to normal basement membrane and basal cell junctions noted in untreated control cases of keratoconus (Fig. 6). Bowman’s layer was also absent in these areas. The keratocytes and endothelium showed evidence of regeneration which was confirmed by electron microscopy (Fig. 7).

At 2½ and 8 months after TKP, the keratocytes and endothelial cells appeared normal. Both specimens showed centrally thinned stroma with focal scar formation. Although irregular epithelial regeneration was present, the epithelial basement membrane was focally absent and hemidesmosomes were deficient in these areas.

Discussion

Cellular and metabolic activities return to normal in rabbits corneas within a few days after TKP using temperatures up to
Fig. 5. One month following TKP. Note degeneration of basal epithelial cells (*), absence of hemidesmosomes (box), and irregularity of epithelial basement membrane (EBM). (x5,500.)

130° C. The extent and rate of healing in human corneas following TKP for keratoconus had not been determined.

Repair of the epithelium following thermal injury (TKP) was somewhat similar to that observed in experimental corneal wounds, including an early leukocytic and macrophagic response which rapidly cleared. Histologic evidence of regeneration was noted 24 hours after TKP and was almost complete 1 month later, except for lack of total regeneration of basement membrane. Although the latter was focally absent in some of our cases, clinical evidence of mild recurrent erosion (superficial punctate keratitis persisting for over 2 weeks) was seen only in one of 29 eyes following TKP. Changes in Bowman’s membrane and epithelial basement membrane may not necessarily be due to TKP, since abnormalities of these structures are characteristic of advanced keratoconus. It is thus difficult to distinguish between the extent of stromal scar observed clinically and histopathologically 1 month following TKP, and those changes due to the disease prior to TKP.

In our study, keratocytes and endothelial cells appeared normal histologically a month following TKP; clinically the corneas appeared clear during the second week. In rabbits, the normalization of keratocytes and endothelial cells occurred at approximately 7 days post-TKP. Re-
Fig. 6. Control (untreated) case of keratoconus. The basal epithelium (E), hemidesmosomes (arrows), basement membrane (*), and Bowman's layer (BL) appear normal. (×47,500.)

Fig. 7. Scanning electron micrograph showing regeneration of endothelium centrally (*) as well as peripherally (arrow). (×35.) Inset shows normal endothelial cells. (×1,750.)
generation of the corneal endothelium and its physiologic function has stimulated considerable interest. Cryothermal injury to the rabbit cornea showed that a 4 to 5 day lag exists between recovery of physiologic endothelial function and histologic recovery.10

Duration of application, size of probe, degree of temperature, and corneal thickness are important factors to consider.5 In our earlier cases, temperatures of 110°C resulted in striate keratopathy which persisted up to 2 weeks, whereas it lasted only 2 to 3 days with a lower temperature of 90°C to 95°C. No permanent changes such as retrocorneal fibrous membrane11 or endothelial transformation were observed.

The results of our study indicate that although TKP induced marked initial inflammatory and edematous changes, the corneas were almost normal after approximately 1 month, except for defective regeneration of epithelial basement membrane.

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REFERENCES